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IS 5404 (1984, Reaffirmed 2005): Methods for Drawing and Handling of Food Samples for Microbiological Analysis (First Revision). UDC 664 [543-058] : 543.9.078



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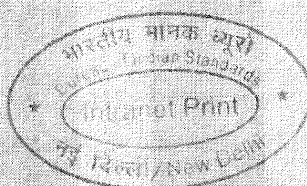
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IS : 5404 - 1984

(Reaffirmed 2005)

Indian Standard
**METHODS FOR DRAWING AND
HANDLING OF FOOD SAMPLES FOR
MICROBIOLOGICAL ANALYSIS**
(First Revision)

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Indian Standard

**METHODS FOR DRAWING AND
HANDLING OF FOOD SAMPLES FOR
MICROBIOLOGICAL ANALYSIS**

(*First Revision*)

Sampling Methods for Food Products and Agricultural
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Indian Standard'

METHODS FOR DRAWING AND
HANDLING OF FOOD SAMPLES FOR
MICROBIOLOGICAL ANALYSIS

(*First Revision*)

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 15 March 1984, after the draft finalized by the Sampling Methods for Food Products and Agricultural Inputs Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 There is a wide variety of foods and accessories of either plant or animal origin available in the market. They include fresh, processed pre-cooked, canned, frozen and dehydrated foods sold in consumer and bulk packages and in containers of different types and sizes.

0.3 All these foods are liable to undergo spoilage by the action of inherent micro organisms or those which gain entry into the products during production, processing and packaging and grow at favourable temperatures during storage. These organisms, may be pathogenic or spoilage types. The pathogenic organisms and toxin-producing types give rise to food poisoning and food-borne infections. The spoilage type may not cause infection but cause spoilage of foodstuffs.

0.4 Microbiological examination of the foods provides information on the hygienic environment of their production and handling, processing efficiency, defects due to microbiol growth and the organisms responsible for the same and presence of specific pathogens and food poisoning organisms.

0.5 This standard was originally issued in 1969 with a view to providing general guidance in regard to the practices to be followed and precautions to be observed in the sampling of different types of foods and in handling of the samples for microbiological analysis. The present revision has been taken up to utilize the experience gained over these years and to streamline the standard with others published in the meantime. In this revised version, general requirements of sampling have been made more exhaustive, the scale of sampling has been modified so as to bring it in line with the corresponding provisions given in other Indian Standards on foods and the

provisions for number of tests and criteria for conformity have been introduced in order to enable a purchaser to decide the conformity or otherwise of a lot to the microbiological requirements of a specification on foods.

0.6 The following important types of foods and food accessories sold in the market (in small or large packings) for human consumption are covered in this standard:

- a) Carbohydrate products (dry and liquid sugar, starch, syrup, honey confectionery and molasses);
- b) Cereals and cereal products (grains, flour and foods containing cereal products);
- c) Eggs and egg products (shell egg, liquid eggs, frozen and dried eggs);
- d) Fermented and pickled foods (sauerkraut, olives);
- e) Fish and fishery products (fish, sardines, shell fish, frozen fish);
- f) Fruits and fruit products (frozen, dehydrated and canned fruits: fruit juices and concentrates);
- g) Meat and meat products including poultry (fresh, ground, canned pickled, cured and dried meat; poultry meat);
- h) Miscellaneous products (carbonated beverages, salad dressings, spices); and
- j) Vegetables and vegetable products (dehydrated, frozen, precooked and canned vegetables, brined, salted and pickled vegetable products).

0.7 Milk and milk foods and special foods, like infant foods, are not included in this standard as the methods of sampling and test for these products are covered in detail in the different parts of IS: 1479* and in the Indian Standards formulated for some of the products individually.

1. SCOPE

1.1 This standard prescribes the procedures for preparation, packaging, storage, preservation and transport of samples of foods for the purpose of microbiological analysis.

*Methods of test for dairy industry (Parts 1 to 5).

2. GENERAL REQUIREMENTS OF SAMPLING

2.1 Sampling shall be carried out by a qualified, trained, experienced and duly authorized person. It is essential that the sample should be representative of the lot to be examined, which may comprise a large number of small packages of materials stored in large containers. Sampling, therefore, requires most careful attention to details if the subsequent analysis is to be of value. Since the samples are required for microbiological analysis, utmost precautions are also necessary to avoid extraneous contamination while drawing and handling the samples and to preserve them in their original condition till they are ready for examination in the laboratory.

2.2 Wherever possible, samples of products in original unopened containers or packages should be drawn and sent to the laboratory without any delay. This will prevent possible contamination of the samples during handling and also help in revealing the true condition of the product as prepared and offered to the public.

2.3 Samples shall be drawn in a protected place without air draught and not exposed to humid conditions, dust or soot, and transferred to sterile containers under aseptic conditions as far as possible.

2.4 The sampling appliances and sample containers shall be clean and sterile.

2.5 All precautions shall be taken to protect the samples, the material being sampled, the sampling instruments and the sample containers against adventitious contamination at the time of drawing the samples, opening sample containers and transferring the samples.

2.6 Since it is impracticable to sterilize certain sampling devices in the field, it is preferable to sterilize such devices in the laboratory and transport them in sterile carrying cases. Where drills, triers, agitators, etc., are used in the field, it is often necessary to sterilize them between samplings. For this purpose, adequate number of sterile sampling devices/equipment should be carried by the sampling authority, where such facilities are not available.

2.7 Hermetically sealed cans shall not be opened under field conditions.

2.8 Each sample container shall be closed with the stopper or sealed airtight after filling with the sample and marked with full details of sampling, batch or code number, name of the manufacturer and other required particulars.

2.9 Samples of dry foods shall be stored in such a manner that the temperature does not vary unduly from the normal temperature. Other samples (either in original packing or transferred to sample containers) shall be held in ice, dry ice or freezing mixture, if so required, according to the nature of the material sampled for analysis with a view to preventing any microbial growth or changes in the microbial flora of the samples during their transport to the laboratory.

2.10 No preservative or bactericidal or fungicidal agent shall be added to samples of foods required for microbiological analysis.

3. SAMPLING APPLIANCES

3.1 The following appliances will be required for drawing samples from different foods:

Stainless steel tubes and glass pipettes for drawing samples of liquid products from cans, barrels or other containers; drills, triers, spoons, scoops, knives and spatula, all made of stainless steel for piercing inside or cutting portions for drawing samples of solid or semi-solid materials; scalpel and knives for scraping the surfaces and cutting portions of fruits, vegetables, meat, etc.

3.2 All sampling equipment shall be perfectly clean and sterile and shall not impart any foreign flavour or odour. They shall be properly sterilized by heating in a hot air-oven at 160°C for not less than 2 hours or by autoclaving for not less than 15 minutes at 120°C and held in suitable containers to prevent re-contamination.

4. SAMPLE CONTAINERS

4.1 Wide-mouth glass jars and bottles of 50 and 100 ml or of larger capacities according to requirements, so that they are almost completely filled by the sample, shall be used. They shall be closed by means of glass stoppers or screw caps. Metal cans with tight-fitting lids and sterile paper bags or polyethylene bags may also be used in some cases according to the nature of product.

4.2 The glass jars and bottles, metal cans, caps, and stoppers shall be perfectly clean and sterile and shall not impart any foreign odour or flavour. They shall be sterilized by heating at 160°C for not less than 2 hours in a hot air-oven or by autoclaving at 120°C for not less than 15 minutes and protected for re-contamination during transfer of sample.

5. SCALE OF SAMPLING

5.1 Lot — All the cans/containers, in a single consignment of the material of the same type and belonging to the same batch of manufacture shall constitute a lot. If a consignment consists of different batches of manufacture, the cans/containers of the same batch shall be separated and each such group shall constitute a lot.

5.2 For ascertaining conformity of the material to the microbiological requirements of a specification, samples shall be tested from each lot separately.

5.3 Eight cans/containers (in addition to those selected from the lot for testing requirements other than microbiological) shall be selected at random from the lot for testing microbiological requirements.

5.3.1 These cans/containers shall be selected at random from all the cases chosen according to Table 1 for processed fruits and vegetables and according to Table 2 for other products. From each selected case, approximately equal number of cans/containers shall be selected.

**TABLE 1 SELECTION OF PACKING CASES FOR
MICROBIOLOGICAL TESTS FOR PROCESSED
FRUITS AND VEGETABLES**

NUMBER OF PACKING CASES IN THE LOT	NUMBER OF PACKING CASES TO BE OPENED
(1)	(2)
up to 150	3
151 to 300	4
301 to 500	5
501 to 1000	6
1001 to 3000	7
3001 and above	8

**TABLE 2 SELECTION OF PACKING CASES FOR
MICROBIOLOGICAL TESTS FOR PRODUCTS OTHER
THAN PROCESSED FRUITS AND VEGETABLES**

NUMBER OF PACKING CASES IN THE LOT	NUMBER OF PACKING CASES TO BE OPENED
(1)	(2)
Up to 8	2
9 to 25	3
26 to 50	4
51 to 100	5
101 to 150	6
151 to 300	7
301 and above	8

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5.4 These cans/containers shall be selected at random from the lot. In order to ensure the randomness of selection, procedures given in IS:4905-1968* may be followed.

5.5 Drawing of Samples for Checking Hygienic Environment and Processing Conditions — Samples of raw materials as well as of the processed and packaged foods may be drawn at various stages of handling and processing in the factory or at any point in the distribution line for microbiological examination to check the hygienic environment and processing efficiency. Such examination should, however, be supplemented with inspection of the premises, processing equipment and personnel hygiene.

6. PREPARATION, TRANSPORT AND STORAGE OF SAMPLES

6.1 In the case of canned foods and small or medium sized packings, the original unopened containers may be taken to the laboratory where the samples shall be withdrawn aseptically for microbiological analysis.

6.2 In regard to foods in bulk or large packings, the samples may be withdrawn from the containers at the factory or the point of sale. The containers selected for sampling, may be opened either by removing the lid or by boring holes on the top with sterile instruments. With appropriate sterile sampling appliances (*see 3*), equal quantities of the material shall be drawn from different parts of the containers, transferred to the sterile sample container and mixed thoroughly. Samples from each of the selected containers shall be drawn and transferred to separate containers.

6.3 Sometimes it may not be possible to draw samples under aseptic conditions. Under such circumstances, the inspector shall inform about it to the laboratory to enable the microbiologist to take aseptic sample from the bulk sample drawn by the inspector.

6.4 In view of the varieties of foods to be handled and the differences in their physical condition, it is not possible to adopt a uniform sampling procedure for all of them. The methods of sampling different types of foods and food products, covered in this standard are given in Appendix A for general guidance.

6.5 The samples of food drawn in the manner described in 2 to 5 shall be transported to the laboratory for testing as quickly as possible, preferably within 24 hours, taking adequate precautions to prevent any change in the original microbial flora of the material.

6.5.1 Dehydrated and dry foods may be shipped and stored without refrigeration but they should not be allowed to absorb any atmospheric moisture. On arrival at the laboratory the samples should be stored in a clean, cool, dust-free place until required for analysis.

*Method for random sampling.

65.2 Samples of unfrozen liquid or semi-solid foods or processed foods in original unopened containers and packages may be transported to the laboratory and stored without any refrigeration. Samples drawn from original packings and transferred to new containers should be held at refrigeration temperatures (4 to 8°C) during transport and storage, but they should not be frozen.

6.5.3 Refrigerated samples of shell eggs should not be exposed to room temperature without a tempering period in order to avoid sweating which facilitates the penetration of bacteria from the shell into the egg albumen.

6.5.4 Meat and meat products, poultry meat and fish should be transported under wet ice refrigeration to avoid dehydration of the surface of the samples.

6.5.5 Samples of frozen food products, either in original packings, or transferred to new containers, should be transported in a solidly frozen condition by holding them in dry ice or freezing mixture in well-insulated containers. On arrival at the laboratory they should be stored in the same condition until required for analysis.

6.6 The samples of foods received in the laboratory should be kept in a frozen condition, in the refrigerator or at room temperature, according to the type of material (see 6.5) till required for examination. The samples should be examined as soon as possible preferably within 6 hours of their receipt.

6.6.1 One of the following methods may be used if the sample received is frozen:

- a) Partially thaw it for 18 hours in a refrigerator at 2 to 5°C.
- b) If the frozen sample can be easily comminuted, proceed without thawing.
- c) With easily thawed material, thaw in an incubator at 35°C for not more than 15 minutes.

6.7 If the samples are in original containers and packages they should be opened carefully using aseptic precautions. In the case of metal cans the top should be cleaned well, and the site of opening sterilized by applying alcohol and flaming. Holes of suitable size should be made by piercing with a sharp instrument and the samples withdrawn with a sterile pipette, tube or spoon. In regard to samples in bottles the corks should be opened aseptically, the mouth flamed lightly and the sample drawn with a pipette.

6.8 In the case of samples of solid or semi-solid materials and liquids (brought in sample containers), which may be easily dissolved or suspended in water, open the lid of the container or screw cap of the bottle

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aseptically, withdraw the required quantity of the material with the help of a sterile spoon or other instrument and transfer to dilution bottles for microbiological examination. Either normal saline, Ringer's solution or phosphate buffer (pH 7.2) may be used as diluents.

6.9 In the case of materials like canned foods, frozen and dehydrated foods, eggs, fish and meat, special procedures should be adopted for removing the samples from the container and bringing them into a suitable form to ensure uniform dispersion of the micro-organisms in the diluent.

6.10 The methods of preparing samples of different types of food for microbiological examination are given in Appendix B.

7. NUMBER OF TESTS AND CRITERIA FOR CONFORMITY

7.1 Eight cans/containers selected from the lot shall be subjected to microbiological examination after incubation as given in 7.1.1.

7.1.1 Four cans/containers shall be incubated at 37°C for not less than 14 days and the remaining 4 incubated at 55°C for not less than 7 days.

7.2 The lot shall be declared as satisfying the microbiological requirements of a specification, if all the cans/containers examined for these requirements meet the corresponding requirements.

8. SAMPLING REPORT

8.1 Complete a detailed sampling report which shall contain the following information:

- a) Name and address of the person collecting the samples,
- b) Name and address of supplier/manufacturer,
- c) Nature of food,
- d) Temperature of the product at the time of sampling,
- e) Means of transporting the samples to the laboratory, and
- f) General remarks, if any.

9. LABELLING OF SAMPLE CONTAINERS

9.1 Immediately before or after the sample is taken, label the container.

9.2 Number the sample label and make a record to identify the same.

9.3 When the sample is taken from a big container, the container should also be marked in the event a subsequent examination is required.

APPENDIX A

(Clause 6.4)

METHODS OF SAMPLING DIFFERENT TYPES OF FOOD FOR MICROBIOLOGICAL EXAMINATION

A-0. GENERAL

A-0.1 Subject to the conditions and procedures specified in 2 to 6, the special methods required to be used for drawing samples of different types of foods in various packings are indicated in this appendix for general guidance.

A-1. SMALL PACKAGES AND CONTAINERS

A-1.1 In the case of all types of foods and food products sold in sealed bottles, tins, cans, cartons, polythene bags and similar containers of small or medium size, as well as canned foods, the original unopened containers should be taken to the laboratory wherever possible. Several representative units should be selected from various parts of the lot according to the directions given in 5.

A-2. LARGE AND BULK CONTAINERS

A-2.1 General Foods — Samples of unfrozen foods and food products in the form of liquids, semi-solids, jellies, powder or grains of uniform consistency held in vats, large tins or cans, and bags, shall be drawn from the containers in the following manner.

A-2.1.1 Open the lid aseptically or pierce holes of convenient size on the top of the container after cleaning and then sterilizing the site by flaming and where not feasible with a suitable antiseptic. Use sterile knife or other sharp instrument. Aseptic precautions should be taken to prevent contamination of the sample from the atmosphere or from the external surface of the container at the site of opening. With the help of sterile spoons, scoops, triers, tubes or pipettes (see 3) equal quantities of the material should be drawn from different parts of the container and transferred to a sterile container. In the case of liquids the material should be properly mixed with the help of a sterile dipper or other instrument before drawing the sample. Samples should be drawn from each of the containers in the lot selected for the purpose and transferred to separate containers. The total amount of sample to be drawn from each container should ordinarily be about 200 g or 200 ml but it may be increased or decreased depending upon the number of tests to be performed on the samples.

A-2.2 Frozen Foods — Open the cans aseptically as indicated in A-2.1.1 and remove a little frozen material from the surface at three different areas using a sterile chisel. Drill three cores at these places from top to bottom of the container preferably with a high speed electric drill. Transfer the drillings to a chilled sample container with a sterile spoon. Drill a fourth hole for examination of odour. The drillings should be kept in a frozen condition by packing in an insulated box with dry ice.

A-2.3 Whole Fruits, Shell Eggs, Fish and Oysters — A representative number of fruits, eggs, fish or oysters should be selected at random from different containers using aseptic precautions. A liberal sample representative of the lot should be taken due to lack of uniformity in the microbial picture from piece to piece. Transfer the samples to sterile jars, cartons or cases. Sterile paper bags may also be used as containers if the samples are handled quickly and examined promptly.

A-2.4 Dehydrated and Dried Foods — Open the containers according to the method described in A-2.1.1. With a sterile spoon draw portions from all parts of each container selected from the lot and transfer the samples to sterile glass jars or cans having tight-fitting lids. In some cases (for example, dried eggs) containing big solid lumps it may be necessary to use a sterile trier or scoop for drawing out cores of the sample. Discard the bottom 2- or 3-cm portion of the material in the trier and transfer the rest of the sample to a sterile jar or can.

A-2.5 Brined and Pickled Vegetables — In the case of brined or pickled vegetables and fermented products containing both solid and liquid components, the brine or pickle liquor or liquid covering the material is required to be drawn for examination.

A-2.5.1 After opening the container or boring a hole on the top (A-2.1.1), insert a sterile stainless steel tube through the opening into the liquid towards the centre of the vegetable or solid mass. Draw out the liquor through a rubber tubing attached to the steel tube at one end and leading to a sterile bottle with suction arrangement for starting the siphon-action at the other end. Withdraw about 500 ml of the liquid into the bottle and from the latter transfer about 200 ml of the sample to a sterile sample container.

A-2.6 Fresh Meat and Meat Products — In order to obtain a true microbiological flora of meats and meat products it is necessary to take representative samples from the surface as well as the deeper portions.

A-2.6.1 Surface Samples — Scrap out thin portions (about 2 mm thick) from various positions on the surface, using a sterile scalpel, knife or spatula, and transfer them with forceps to a sample container.

A-2.6.2 Deep or Core Samples — In regard to solid pieces of meat the surface should be sterilized by searing. Then cut into the centre at the area with a sterile knife and remove the sample. In the case of sausages the surface may be scored with a knife, the sausage broken by hand and core samples removed with a sterile knife or scalpel. If a particular spot or area is obviously spoiled or discoloured, a sample of spoiled material should be taken and for comparison an apparently unspoiled portion from a similar location on the product should also be drawn and transferred to separate containers.

A-2.6.3 Poultry Meat — In the case of dressed and eviscerated poultry, select the birds at random from the containers to be representative of the lot. In regard to cut-up poultry, select parts from different portions to be representative of the lot. Transfer the samples to sterile containers or wrap in sterilized paper, cloth or polythene bags of convenient size.

APPENDIX B

(Clause 6.10)

METHODS OF PREPARING SAMPLES OF DIFFERENT TYPES OF FOOD FOR MICROBIOLOGICAL ANALYSIS

B-1. CANNED FOODS

B-1.1 Preliminary Observation — Record the particulars of the sample, namely, its size, make of container, code marks, etc. Note the condition of the can, mechanical defects, perforations, rust spots, dents and can abnormalities.

B-1.2 Preparation of cans for Opening — Clean the can with soap and water. If the can is greasy petroleum ether, alcohol or naphtha may be applied at the site of opening. The cans should be preferably opened at a suitable place so as to preserve the packers code and also avoid disturbance of the seaming compound at the packer's end of the can. The site of the opening may be sterilized by holding the can over the flame of a burner and distributing the heat with a circular motion over the previously cleaned top. The burner should not be played down on the top of the can as this will result in a concentration of heat at the top causing scorching of the material and it might lead to spurting of the contents when the opening is made. If the containers are badly swollen they should not be flamed but sterilized by applying mercuric chloride solution (0.2 percent) or 70 percent alcohol.

B-1.3 Opening of Container — If the can contains solid or semi-solid products, cut a circular disc around a central puncture using a sterile can opener. In the case of liquid products, cut a hole about 1.5 cm in diameter with a sterilized tapered punch.

B-1.4 Removal of Sample — Solid products may be removed by using sterile cheese triers, spoon or cork-bore. In the case of semi-solid and liquid products the samples may be drawn by using sterile untapered glass tubes or pipettes.

B-1.4.1 The material may be transferred to dilution bottles or directly into different media in test-tubes or petri dishes for determining numbers and types of micro-organisms in the canned material.

B-1.4.2 In the case of concentrated acidic products, for example, tomato paste or fruit pulp, the spoilage may be highly localized. The top bottom and side of the product should be carefully examined separately for growth of micro-organisms.

B-1.5 Incubation Test — In incubated tests for determining keeping quality and sterility of the products, sound cans only shall be used.

B-2. CARBOHYDRATE PRODUCTS (SUGARS, STARCH, SYRUP, ETC)

B-2.1 Open the container aseptically. Remove 20 g of the sample into a sterile 150-ml conical flask, add 100 ml of sterile distilled water, dissolve and shake thoroughly to mix the contents. Aliquots may be drawn from the solution for microbiological analysis.

B-3. CEREALS AND CEREAL PRODUCTS

B-3.1 Remove any dust from the surface of the package with a slightly moistened cloth and then aseptically open the package and remove the sample. Make a representative composite sample from several of the packages.

B-3.2 In order to obtain uniform and accurate results it is necessary to use comparatively large samples and to have them in a rather finely divided state, for example, flour or meal. In dry or solid cereal products distribution of the bacterial population is frequently not uniform, hence the necessity of using comparatively large amount to obtain representative samples.

B-3.3 In smaller particles bacteria are washed off into the dilution water easily. The process may be aided by the abrasive action of sand, glass beads, etc, incorporated in dilution blanks. If the sample is in the form of a flour it is tested without further treatment, but if it is coarse, the particle

size of the sample should be reduced to approximately that of a coarse meal by grinding, with aseptic precautions, in a sterile mortar and pestle, or other sterile grinding equipment. In the case of whole grain, like rice, grinding is not necessary. In grains, like wheat, with creases and irregular surfaces, it is necessary to grind them before testing.

B-3.4 After thoroughly mixing the prepared samples., weigh approximately 11 g in a sterile aluminium boat or on a piece of sterile paper. Transfer to a dilution bottle with a stopper, containing 99 ml of sterile water and approximately 10 g of purified sterile sand. Shake vigorously for at least 2 minutes. Allow to stand for 2 or 3 minutes or until most of the larger solid particles have settled to the bottom, This constitutes the primary dilution.

B-4. EGGS AND EGG PRODUCTS

B-4.1 Shell Eggs -Wash each egg with a brush using soap and water, drain and immerse in 70 percent alcohol for 10 minutes. Remove from alcohol, and drain. Grasp egg by blunt or air-sac end and puncture a hole in the opposite and with a sterile scalpel or forceps. Flame opening before removal of contents. Invert opened egg over a sterile crucible triangle placed on a tripod. Place sterile wide-mouth bottle beneath egg and force the contents out by gently applying heat with a Bunsen Aame to the air-sac end. Shake the contents of the container or beat with a sterile spoon or electric mixer until the sample is homogeneous.

B-4.1.1 Egg white or egg yolk may be separated, if required, by the use of a sterile commercial separator or by the use of sterile spoon. The yolk may be freed of excess egg white by rolling it on a sterile towel.

B-4.2 Liquid Eggs — Thoroughly mix the contents of the sample jar until it is free of any lump and is homogeneous. If the sample is egg white, it should be free of lumps of thick white. Care should be taken in the mixing to avoid excessive foaming of the sample.

B-4.3 Frozen Eggs-Thaw the contents of the sample container as rapidly as possible. Use of running tap water is recommended with bottle or jar two-thirds submerged. Rotate the container frequently until, the material is thawed. Open the container using aspectic precautions and thoroughly mix the contents to get uniformity. Alternatively, thaw overnight in a refrigerator.

B-4.4 Dried Egg — Remove the sample containers of whole egg and yolk from the refrigerator and allow them to come to room temperature. If the powder is warm, better dispersion in the dilution blanks will be obtained. Open sample containers under aseptic conditions and thoroughly mix with a sterile spoon. If the pieces of flake albumen are thick and of

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large size they should be crushed in a sterilized mortar until the pieces are smaller than 0.6 cm. The required quantity of the material may be transferred to dilution blanks.

B-5. FISH, SHELL-FISH, OYSTERS

B-5.1 Scrape off excessive growth and loose material from the fish and scrub with a brush in running water of known purity until shells are clean and free of all mud. In the case of unopened shell-fish, rinse it in clear water and dry in air. Open the shell fish with a sterile knife, drain the shell liquor into a sterile bottle and remove the shell using aseptic precautions. Cut the body of the fish into small pieces with a sterilized knife and transfer the pieces into the bottle containing shell liquor. Add sterile glass beads or some fine sand. Shake vigorously. Add equal amount of sterile distilled water, shake vigorously and allow the sand or glass beads and coarse muscle particles to settle down. Use the supernatant liquid for plate count or microscopic examination.

B-6. FRUITS AND VEGETABLES

B-6.1 Fruit Concentrates and Juices— While opening the container examine for signs of fermentation and mould growth. Mix the contents well by inverting the container or by stirring to obtain good distribution and draw the sample aseptically. Concentrates should be reconstituted to the original strength using sterile distilled water. For special examination (coliform contamination) the reconstitution should be made with sterile sodium hydroxide solution (0.6 percent) to adjust the pH to about 5.5 to 6.0.

B-6.2 Frozen Fruits— Hold the packages of frozen fruit at room temperature for 1 to 2 hours to temper before opening. While the fruit is still partially frozen cut portions from various parts of contents of the package using a sterilized scalpel. Weigh 50 g of fruit or syrup into a sterile blender, add 450 ml sterile water and blend for 2 minutes.

B-6.3 Frozen Vegetables (Peas, Lima Beans, Cut Corn, Whole or Regular Cut Green Beans Etc)— Break the sample, if not loose-frozen, into small units by tapping unopened package sharply against a table edge or by sharply straking with a dull instrument, being careful not to break open the package. Open the package and remove sample with sterile spoon or knife taking the sample from various parts of the broken out package, for example, from centre and corners.

B-6.3.1 Weigh a 50-g sample into a sterile mechanical blender cup. Add 450 ml of sterile water and blend contents for 2 minutes. Allow the sample to stand for 2 to 3 minutes to permit foam to subside. Resuspended sediment by swirling and pipette 10 ml of liquid into a 90-ml sterile water blank.

B-6.4 Frozen Spinach, Cauliflower, etc — Allow the package to defrost partially by standing at room temperature for $1\frac{1}{2}$ to 2 hours or take small pieces of frozen material and cut out with a sterile knife, scalpel or scissors. Prepare the samples from various portions of the package taking care to select different parts of the vegetable. Add sterile water and blend for 2 minutes.

B-6.5 Dehydrated Fruits

B-6.5.1 Apples, Apricots, Peaches, etc — Select 20 pieces and weigh into a sterile screw-cap jar and add 200 ml of sterile water. Allow the jar to stand for 30 minutes at room temperature and then shake by hand or by machine for 1 to 3 minutes.

B-6.5.2 Prunes, Figs and Dates — Select 10 fruits and cut in two with a sterile knife. Weigh into a sterile jar and add 100 ml of sterile water. Allow to stand and then shake.

B-6.5.3 Raisins, Currants, Cherries — Weigh about 100 pieces of fruit into a sterile jar. Add 200 ml of sterile water. Allow to stand and shake.

B-6.6 Dehydrated Vegetables

B-6.6.1 Leafy Vegetable — Weigh 10 to 20 g into a sterile bottle or flask. Add 190 ml of sterile water. Allow to rehydrate for 30 minutes at refrigeration temperatures and shake vigorously for 2 minutes.

B-6.6.2 Roots and Solid Vegetables — Weigh 10 to 20 g into a sterile bottle or flask and add 190 ml of sterile water. Allow to rehydrate for 30 minutes at refrigeration temperature and shake for 2 to 3 minutes.

B-7. MEAT AND MEAT PRODUCTS

B-7.1 Emulsify 11 g of the sample in 99 ml of a sterile diluent in a sterile mechanical blender cup. Blend for 3 minutes only to avoid overheating of the sample.

B-7.1.1 Shake the sample (3 minutes) using a mechanical paint mixing machine or other shaking device or by hand.

B-7.2 Canned Meats- Use the same method as for canned foods (see B-1).

B-8. POULTRY

B-8.1 Poultry Meat — Place the samples in warm air where the sample can thaw or warm quickly. Do not allow the surface to become dry.

B-8.1.1 Use small cotton swabs (on round wooden applicators) for sampling surface area of birds or use a convenient template (waxed paper gasket) for sampling the surface area.

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B-8.2 Cut-Up Poultry — Arrange selected poultry parts for ready access for cutting. Open the plastic bag to receive the sample. With sterilized tongs transfer a part to the plastic bag. Add sterile saline water to the nearest 100 ml approximating 3 times the mass of the pieces of chicken. Squeeze about half of the air from the bag, gather the open end and twist firmly leaving considerable slack in the bag. Shake the bag vigorously.

B-8.3 Dressed and Eviscerated Poultry — Select a sterile template with sterile forceps, place firmly against the skin surface of the breast of the bird and hold with figures placed on the outside edge of the template. Thoroughly swab the entire skin surface exposed in the opening of the portion, rolling the swab to expose all surfaces.

B-8.3.1 Insert the swab in a dilution bottle about 5 cm within the neck. Break the lid off by bending the applicator sharply against the glass dropping the cotton tip into the dilution water.

B-9. SALAD DRESSINGS

B-9.1 Mix the sample thoroughly using a sterile spatula. Liquid emulsions should be vigorously shaken 25 times before making dilutions. Weigh 20 g of the material into 10 ml of sterile water. Agitate the contents thoroughly for 10 minutes either in a shaker or by hand. Stabilizing agents like polysorbate 80 may be used if necessary.

B-10. SPICES

B-10.1 Transfer weighed quantity of spices from the container into a dilution bottle using aseptic precautions. Shake the bottle thoroughly to free the micro-organisms from the surface of the spices and distribute them uniformly in the diluent. Allow coarse particles to settle down and use the supernatant liquid for microbiological examination.

INTERNATIONAL SYSTEM OF UNITS (SI UNITS)

Base Units

Quantity	Unit	Symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole	mol

Supplementary Units

Quantity	Unit	Symbol
Plane angle	radian	rad
Solid angle	steradian	sr

Derived Units

Quantity	Unit	Symbol	Definition
Force	newton	N	1 N = 1 kg. m/s ²
Energy	joule	J	1 J = 1 N.m
Power	watt	W	1 W = 1 J/s
Flux	weber	Wb	1 Wb = 1 V.s
Flux density	tesla	T	1 T = 1 Wb/m ²
Frequency	hertz	Hz	1 Hz = 1 c/s (s ⁻¹)
Electric conductance	siemens	S	1 S = 1 A/V
Electromotive force	volt	V	1 V = 1 W/A
Pressure, stress	pascal	Pa	1 Pa = 1 N/m ²

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